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Adjusting tropical marine water quality guideline values for elevated ocean temperatures

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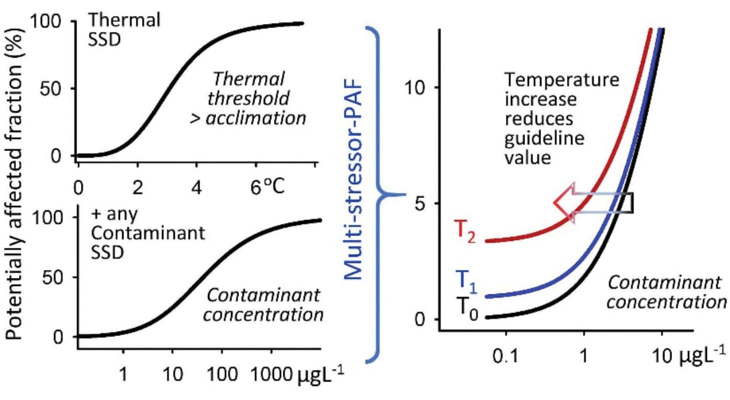
ABSTRACT

Increased frequency of summer heatwaves and poor water quality are two of the most prevalent and severe pressures facing coral reefs. While these pressures often co-occur, their potential risks to tropical marine species are usually considered independently. Here we extended the application of multisubstance-PAF (ms-PAF) to a non-chemical stressor – elevated sea surface temperature. We then applied this method to calculate climate-adjusted water quality guideline values (GV) for two reference toxicants, copper and the herbicide diuron for tropical marine species. Firstly, we developed a species sensitivity distribution (SSD) for thermal stress based on published experimental data for 41 tropical benthic marine species, using methods adapted from water quality GV derivation. This enabled quantitative predictions of community effects as temperatures exceeded acclimation values. The resulting protective temperature values (*PTx*) were similar to temperatures known to initiate coral bleaching, and are therefore relevant for application in multi-stressor risk assessments. The extended ms-PAF method enabled the adjustment of current water quality GVs to account for thermal stress events. This approach could be applied to other ecosystems and other non-contaminant stressors (e.g. sediment, low salinity, anoxia and ocean acidification), offering an alternative approach for deriving environmental GVs, reporting and assessing the risk posed by multiple stressors.



INTRODUCTION

Tropical marine ecosystems are under increasing pressure from ocean warming, and stressful warming events can occur simultaneously with poor water quality to accelerate the decline of coral reefs[1,2]. Maintaining contaminant concentrations below water quality guideline values (GV) is one objective of water quality management programs, intended to improve the resilience and recovery of reefs to thermal bleaching of corals[3,4]. However, current GVs are derived from single-chemical ecotoxicological experiments performed under optimal conditions and do not account for additional stressors that can affect the toxicity of contaminants[5-8]. There are many studies that demonstrate the influence of temperature on the sensitivity of marine ectotherms to contaminants, and increases in metal toxicity above a thermal optimum is a common example[7-10]. As temperatures in tropical coral reef waters have already increased by around 1 °C since the industrial revolution[11], and further 2 – 3 °C increases are predicted by 2100[11,12], it is crucial to consider adjustments to water quality GVs ("climate-adjusted-thresholds") for heatwave conditions that will become more frequent and longer in duration as global temperatures increase[13].

Understanding the effects of temperature on marine water quality GVs requires data representative of marine communities (rather than single species), but there are currently no empirical datasets that quantify the combined risk of thermal stress and any contaminant to marine communities. Contaminant GVs are usually based on species sensitivity distributions (SSD)[14]; that describe the relationship between the potentially affected fraction (PAF) of multiple species relative to contaminant concentration. SSDs developed from species and toxicity test conditions relevant to different climates often show differences in sensitivity to a range of contaminants [5,15,16]. For example, Zhou et al.[7] modelled the influence of temperature on SSDs for three metals over 10 and 15 °C temperature ranges, and showed the concentration protective of 90% of species reduced by up to an order of magnitude for warmer climate species, suggesting a 10-fold safety factor to protect communities across wide temperature ranges. However, those SSDs were each developed from optimal temperature tests and do not address the case where organisms are exposed to contaminants outside their normal thermal ranges.

Generating SSDs that account for specific contaminants at multiple temperatures is presently limited by inadequate experimental data[7]. An alternative approach is to estimate the PAF of the combined effects from individual SSDs of chemicals with an SSD derived for thermal effects, using the multi-substance potentially affected fraction (ms-PAF) method[17] (re-named here "multi-stressor-PAF" to indicate that chemical and non-chemical stressors are being combined). This method is mainly used to predict the combined effects of chemical mixtures from individual chemical SSDs by applying well-established models of joint action[18,19], such as the independent action (IA) model (also known as the response addition model), applied where the chemicals do not have the same mode of action [19]. Originally intended to address mixture toxicity for single species, their application to predict the ecological risk of complex chemical mixtures by applying these to multiple SSDs has been proposed[17], critically reviewed[20], and applied to mixtures of contaminants with up to 10 different modes of action[21]. While limited by assumptions that are not always met, ms-PAF rarely overestimates combined effects and represents the most used and validated "bottom-up" approach to predict the effects of multiple contaminants on communities from single contaminant data[17,21]. To extend this application to include thermal stress on marine communities, SSDs are needed that relate the PAF of a marine assemblage to temperature stress (there are already many aquatic SSDs for individual contaminants[22]). Like metals and many contaminants, thermal stress acts on multiple pathways simultaneously[9] and these will differ between species; therefore, temperature stress need not be treated differently to most contaminants with respect to deriving SSDs. So far, thermal SSDs have been described for freshwater fish[23] and molluscs[24], as well as mostly temperate coastal species across a 15 °C range (from 5 to 20 °C)[25].

However, thermal SSDs for tropical marine species are likely to be distinctive as they are exposed to narrower temperature ranges, leading to low temperature stress thresholds above species optima for many taxa[26,27].

Here, we developed the first thermal SSD for tropical species based on temperature stress thresholds of 41 species across 12 phyla. This allowed the derivation of temperatures below which 99, 95, 90 and 80% of tropical marine species respectively should be protected. We then explore the use of the ms-PAF IA model to combine temperature and chemical SSDs to derive climate-adjusted water quality GVs.

MATERIALS AND METHODS

SSD for thermal stress. Since SSDs represent a community response to stress and thermal stress acts on multiple pathways, the development of a thermal SSD for marine communities requires temperature stress data for multiple species across diverse phyla[25]. A literature search was conducted to identify studies that had quantified the effects of elevated temperatures on benthic marine species associated with tropical coral reefs. The Web of Science and Google Scholar were used to search for literature published after 1980 using the following terms: (aquatic OR marine OR estuar* OR coral) AND (temperature OR thermal OR temp*) AND (ecotoxic* OR toxic*) AND (tropical OR sub- tropical OR subtropical), and supplemented with papers cited in recent reviews[2,28]. Only studies with species that occur in the tropics and on coral reef ecosystems were included, and only tests performed at 18 °C or higher, which has been identified as the minimum temperature for coral reef growth[29], were considered. In order to apply ms-PAF to estimate the joint effects of multiple stressors, SSDs need to be derived from consistent endpoint metrics (e.g. LC50) and exposure durations (either acute or chronic)[17]. The contaminant SSDs (below) were derived using Australian and New Zealand water quality guideline (ANZ) methods[30,31], so the thermal SSD was derived using the same methods. Temperature stress data were eligible for consideration if the source included adequate descriptions of: (i) the temperatures applied and exposure period; (ii) replication; and (iii) the statistical method or model used to determine effect. Studies were only included if they: (i) reported ecologically-relevant endpoints (e.g. mortality, growth, reproduction) and (ii) were conducted over “chronic” exposure durations (as defined in Warne et al.[31]). The data quality assessment process was formalised by answering 18 questions on how the data were generated (e.g. test organism, experimental, design and statistical analysis) based on the information provided in the articles[30,31] (Table S1, Supplementary Material). Only “acceptable” quality experimental data (score ≥50%[31]) were included in the thermal SSD to ensure reliable and consistent temperature stress values. Most of the publications reported multiple endpoints; however, the dataset was reduced to obtain a single threshold value to represent the sensitivity of each species in the SSD[31].

Metrics for temperature stress have previously included the temperature at which 50% of organisms are killed (ET50), or the time taken to reach 50% mortality[25,32]. For consistency with existing contaminant SSDs, only thermal effects data that caused a 10% ecologically relevant effect (ET10) or no observed effect (NOET) for each species were used to create the thermal SSD in the present study. We defined the temperature threshold (TTx) for each species as the maximum temperature above acclimation at which negative affects did not occur as follows:

$$TTx = Tx - Ta \tag{1}$$

Ta (°C) is the experimental acclimation temperature (even for short acclimation periods). Most experiments applied few temperature temperatures and Ta was assumed optimal. Tx (°C) is the highest temperature exceeding the Ta where there is no statistically significant effect ($P > 0.05$) on species x (i.e. NOET) or the effect is not greater than 10% (i.e. ET10). If the NOET was equivalent to the acclimation temperature (Ta), the data were excluded, as this provided no information to identify a thermal effect threshold above acclimation.

For consistency with the copper and diuron SSDs (below), the thermal SSD was derived using the Burrlioz 2.0 software[33]. This software applies the Burr type III statistical distribution that best fits the effect TTx data of multiple species, and calculates the potentially affected fraction of species in a community (PAF (%)) according to the following equation:

$$PAF\ (\%)_{Burr\ III} = \frac{100}{\left[1+\left(\frac{b}{TTx}\right)^{c}\right]^{k}} \tag{2}$$

where b, c and k are location and shape parameters of the selected distribution. Burrlioz 2.0 calculated temperatures for four levels of ecosystem protection, i.e. the protective temperature (PTx) for 80%, 90%, 95% and 99% of species in a community/ecosystem (PT80, PT90, PT95 and PT99, respectively). Alternative SSD calculation methods (e.g. gamma, lognormal, log-log) derived almost identical PTx values (Table S2). This thermal SSD, derived from chronic data, will be relevant to extended periods of increased sea surface temperature (e.g. summer heatwave conditions of several weeks), but not short-term temperature fluctuations (e.g. pulsed releases of heated water from industry) nor to the longer-term increases to basal sea surface temperatures associated with climate change.

SSD for copper and diuron: Ecotoxicity data to generate the SSD for copper (26 marine species from 8 phyla) were obtained from the Australian and New Zealand water quality guidelines [34]. These data were originally collated and used to derive the copper guideline values in 2000. Ecotoxicity data to generate the SSD for diuron (20 marine species that belonged to six phyla) were obtained from recently proposed water quality guidelines[35]. The SSDs for these contaminants were also derived using Burrlioz 2.0[33].

ms-PAF to predict the joint effects of thermal stress and contaminants using the Independent Action model. The independent action (IA) model of joint action is used for stressors that have different modes of action but do not interact at the target site of toxic action[17,19]. IA was chosen as the most appropriate model of joint action to estimate the combined effect of temperature stress on each of the two contaminants as the modes of action of all three stressors are likely to differ (including between species) and because IA is considered more conservative (generally predicts lower joint action) than the alternate concentration addition approach[17]. Furthermore, IA was previously shown to be a good predictive model for single species responses to copper and diuron in combination with thermal stress[28,36,37]. Using the IA model of joint action, the "multiple stressor" potentially affected fraction of a community ($ms\text{-}PAF_{IA}$) was calculated by combining the PAF of each stressor[17] as follows:

$$ms\text{-}PAF_{IA} = PAF_A + PAF_B - (PAF_A \times PAF_B) \quad (3)$$

where PAF_A and PAF_B are the potentially affected fractions from two stressors with different modes of action that do not interact (e.g. temperature and copper or temperature and diuron), scaled from 0 (0% of species in the community affected) to 1 (100% of species in the community affected).

RESULTS

Data contributing to the thermal SSD. The literature search identified thermal stress data for 41 species belonging to 12 phyla (Table S3), which met the minimum data requirements to derive an SSD[30,31]. The species included tropical habitat building corals, crustose coralline algae, calcifying green algae, seagrasses, reef-associated invertebrates including foraminifera and sea urchins, as well as benthic diatoms and coral symbionts (Table S3).

Thermal SSD and guideline values for thermal stress and contaminants. The thermal threshold (*TTx*) values ranged from 1.0 – 6.3 °C (Table S3). The curve fit was not bimodal and there was no apparent relationship between taxa and threshold. For example, some coral species had *TTx* values as low as 1 – 2 °C above acclimation, while the survival of other coral species were only affected at 6 °C above acclimation (Figure 1, Table S3). There was no correlation between the acclimation temperature *Ta* and the *TTx* in this dataset (R^2 = 0.0125, Figure S1). The fit of the Burr III distribution to the data, combined with the amount and types of ecotoxicity data available (Table S3), resulted in a set of 'very high reliability'[31] *PTx* values (Table 1). These *PTx* values are the equivalent of *PCx* values for contaminants, such that at least 99% of species will be protected from adverse chronic effects provided the temperature was not greater than 0.83 °C above the acclimation temperature and no contaminants were present (Table 1). Similarly, at least 95% of species would be protected from adverse chronic effects provided the temperature was not greater than 1.4 °C above the acclimation temperature and no contaminants were present.

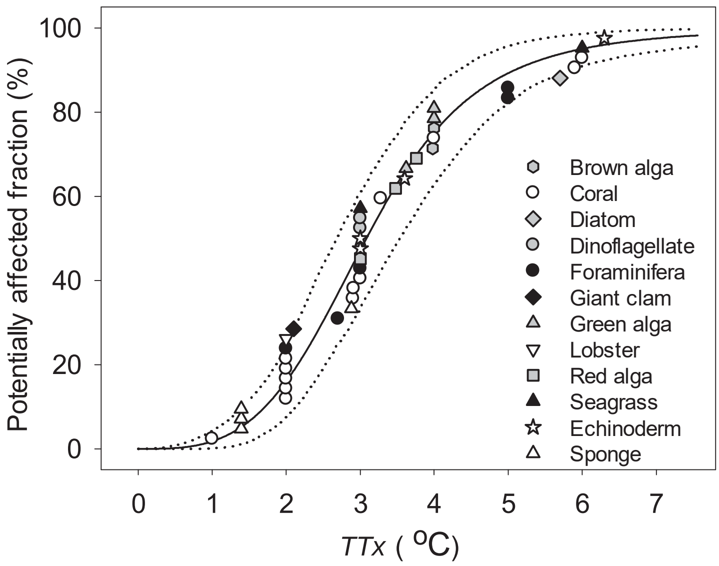


Figure 1. Species sensitivity distribution (SSD) of the thermal threshold data (*TTx*) used to derive protective temperature values (*PTx*). SSD was fitted with the Burr III function using Burrlioz 2.0[33] and replotted using SigmaPlot 13 (Systat Software Inc.). The dashed lines are the 95% confidence intervals for the SSD.

The diuron and copper SSDs (Figure S2, Table S4) generated 'very high reliability' *PCx* values (Table 2) based on the fit of the Burr III distribution to the data combined with the amount and type of ecotoxicity data available[31]. The *PCx* values for diuron were identical to those previously published by Warne et al.[35], while the *PCx* values for copper were very similar to those in the Australia and New Zealand national water quality guidelines (0.3, 1.3, 3, 8 μg L^{-1} for Cu PC99, PC95, PC90, PC80 respectively[34]). These minor differences in *PCx* values are due to updates in Burrlioz software since the copper GVs were originally derived in 2000 (the same data were used to derive the previous GVs and those generated in this study).

Table 1. The protective temperatures *PTx* (% CI range) for elevated temperature exposure in the absence (SSD, Figure 1) and in the presence of elevated concentrations of contaminants (Figure 2). Concentrations of copper and diuron equivalent to their *PCx* values were applied resulting in identical adjustments to *PTx* for each (or any) contaminant. Grey percentages in brackets indicate the % change in the *PTx* values compared to increased temperature alone (no contaminant present).

Thermal stress	PT99 (°C)	PT95 (°C)	PT90 (°C)	PT80 (°C)	PT50 (°C)
No contaminants	0.83 (0.50—1.4)	1.4 (1.0—1.8)	1.7 (1.4—2.1)	2.2 (1.8—2.5)	3.1 (2.6—3.5)
+PC99 Cu or diuron	-	1.3 *(8%)*	1.7 *(2%)*	2.1 *(4%)*	3.1 *(1%)*
+PC95 Cu or diuron	-	-	1.4 *(18%)*	2.0 *(10%)*	3.0 *(3%)*
+PC90 Cu or diuron	-	-	-	1.8 *(20%)*	2.9 *(6%)*
+PC80 Cu or diuron	-	-	-	-	2.7 *(13%)*

Table 2. Protective concentrations *PCx* (95% CI range) for copper and diuron exposures (SSDs in Figure S2) at the acclimation temperature (*Ta*) of each species. The table also includes the influence of elevated temperature on *PCx* values (μg L^{-1}) for copper and for diuron exposures. Grey percentages in brackets indicate the % change in the *PCx* values due to temperature exposure above the *Ta*. As temperatures increase to 1.5 °C above acclimation no adjustment is possible for PC99 and PC95 as > 5% of species are predicted to be affected by temperature alone (Table 1).

Copper	PC99 (μg L^{-1})	PC95 (μg L^{-1})	PC90 (μg L^{-1})	PC80 (μg L^{-1})	PC50 (μg L^{-1})
Ta	0.29 (0.024—2.2)	1.4 (0.44—5.4)	3.0 (1.2—9.4)	7.4 (3.0—20)	42 (20—108)
Ta +0.5 °C	0.25 *(14%)*	1.4 *(0%)*	3.0 *(0%)*	7.4 *(0%)*	42 *(0%)*
Ta +1.0 °C	-	0.90 *(36%)*	2.4 *(20%)*	6.6 *(11%)*	40 *(5%)*
Ta +1.5 °C	-	-	1.0 *(67%)*	4.7 *(36%)*	35 *(17%)*
Ta +2.0 °C	-	-	-	1.3 *(82%)*	25 *(40%)*
Ta +2.5 °C	-	-	-	-	13 *(69%)*
Diuron	PC99 (μg L^{-1})	PC95 (μg L^{-1})	PC90 (μg L^{-1})	PC80 (μg L^{-1})	PC50 (μg L^{-1})
Ta	0.43 (0.079—0.77)	0.67 (0.39—1.1)	0.86 (0.70—1.4)	1.20 (0.99—2.1)	2.4 (1.8—2.4)
Ta +1.0 °C	-	0.59 *(12%)*	0.80 *(7%)*	1.13 *(6%)*	2.3 *(4%)*
Ta +1.5 °C	-	-	0.61 *(29%)*	1.0 *(17%)*	2.2 *(8%)*
Ta +2.0 °C	-	-	-	0.66 *(45%)*	1.9 *(21%)*
Ta +2.5 °C	-	-	-	-	1.4 *(42%)*

Predicting the influence of thermal stress on diuron and copper guideline values using ms-PAF: The influence of increasing thermal stress on the copper SSD, predicted by the IA joint action model[17] was to shift the SSD vertically above the standard SSD based on toxicity data derived at acclimation temperatures (Figure 2A). This in turn affected *PCx* values. For example, at a temperature of 1 °C above acclimation (*Ta* +1 °C) the PC95 for copper decreased to 0.9 µg L^{-1} (a reduction of 36%, Table 2) in order to still protect 99% of species. The shift in *PCx* was larger for low *PCx* values and at increasing temperatures above acclimation *(TTx)*. As temperatures increase to 1.5 °C above acclimation no adjustment to PC99 and PC95 values is possible as > 5% of species are predicted to be affected by temperature alone (Table 1). At 1.0°C above acclimation an adjustment of the copper PC95 of 36% is suggested but no adjustment of the PC99 is required as this threshold has already been reached at an increased temperature of 0.83°C (from the SSD and Table 1). The SSDs converged at high copper concentrations (> 10 µg L^{-1}) as the metal started to have a greater influence on the percentage of affected species (up to *Ta* +2.5 °C).

Increasing thermal stress was also predicted to shift the diuron SSD vertically above the SSD based on toxicity data derived at acclimation temperatures (Figure 2B). At *Ta* +1 °C the PC95 for diuron decreased to 0.59 µg L^{-1} (a reduction of 12%, Table 2). The greatest predicted influence of temperature on diuron GVs occurs as the *PCx* decreases (i.e. greater proportional effect at PC95 than PC90) and at higher temperatures. For example, the PC80 for diuron would need to be reduced from 1.20 to 0.66 µg L^{-1} at *Ta* + 2 °C in order to still protect 80% of species from this combination of herbicide and thermal stress (Table 2).

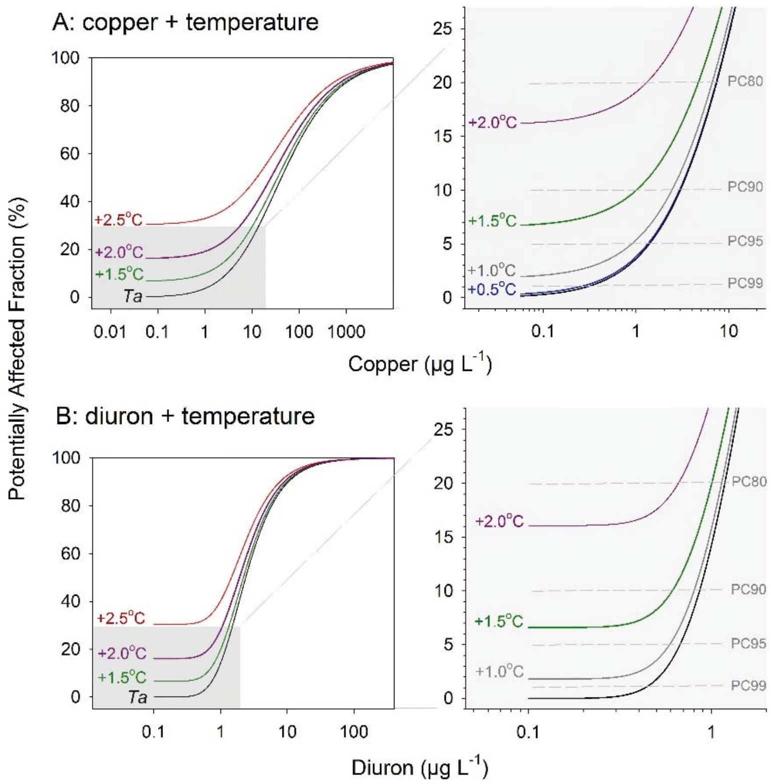


Figure 2. Species sensitivity distributions (SSDs) for copper (A) and diuron (B) at acclimation temperatures (*Ta*, black curve), and at temperatures greater than the acclimation temperature (*TTx*). The left plot shows full-scale SSDs while the right plot is an expanded view of grey areas in the left-hand plots. Adjustments to water quality guideline values (PC99 – PC50) are shown in Table 2.

Predicting the influence of contaminants on the thermal stress protection temperature values using ms-PAF: Low concentrations of copper and diuron caused minor shifts in the thermal SSD (Figure 3), and only a small predicted effect on *PTx* values (Table 1). Concentrations of copper and diuron equivalent to their *PCx* values were applied resulting in identical adjustments to *PTx* for each contaminant. The presence of any contaminant at its *PT95* concentration would have the same influence of *PTx*. For example, PT95 reduced by the same amount from 1.4 to 1.3 °C when co-exposed to the PC99 concentrations of copper (0.29 μg L^{-1}) or diuron (0.43 μg L^{-1}) (Table 1). The influence of contaminants on *PTx* increases at higher concentrations. For example, the PT80 for thermal stress was reduced from 2.2 to 1.8 °C when co-exposed to the PC90 concentrations of copper (3.0 μg L^{-1}) or diuron (0.86 μg L^{-1}) (Table 1).

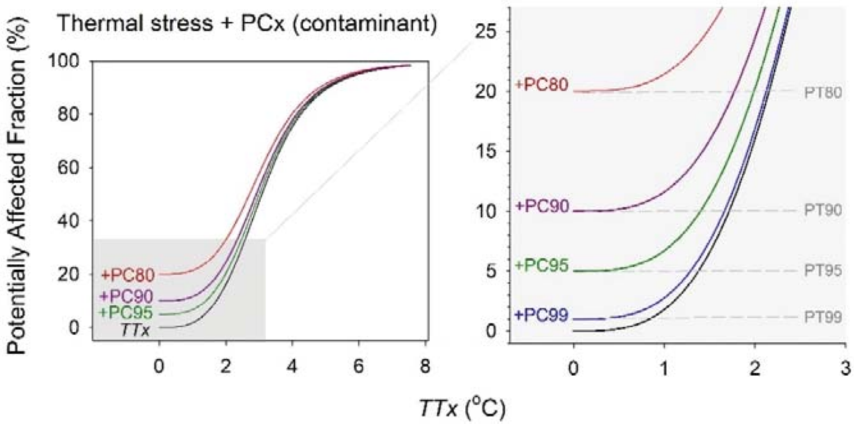


Figure 3. Species sensitivity distribution (SSD) for thermal stress at temperatures above acclimation (*TTx*) (black curve), and shifts in the thermal SSD at PC99, PC95, PC90 and PC80 concentrations for any contaminant (values for copper and diuron can be found in Table 2). The left plot shows full scale SSDs while the right plot is an expanded view of grey areas in the left-hand plots. Adjustments to suggested thermal stress guideline values (PT99 – PT50) as contaminant concentrations increase are shown in Table 1.

DISCUSSION

Water temperature thresholds and temperature stress guideline values. The generation of a thermal stress SSD for tropical species from ET10/NOET data allowed us to derive thermal limits (e.g. PT95 = 1.4 °C), below which a proportion (e.g. 95%) of the community would be protected from deleterious effects of thermal stress. This result is consistent with the hazardous temperature increase (HTI5) of <2 °C (protective of 95% of species and equivalent to our PT95) calculated by de Vries et al. (2008)[25] for 50 temperate aquatic species based on modelled 10% mortality data. The *PTx* values could also be compared with thresholds for thermal bleaching of corals where photosynthetic symbionts are lost (often leading to colony mortality) during periods of high sea surface temperatures (SST)[38,39]. The analysis of many years of bleaching and SST data has led to the development of bleaching threshold indices such as Degree Heating Weeks (DHW), a measure of thermal stress accumulated through time, calculated by integrating temperatures at least 1 °C above the mean monthly maximum[40]. For example, 4 weeks of temperatures 1.5 °C above the mean monthly summer maximum equals 6 DHW (e.g. 4 x 1.5°C-weeks), and values of between 4 and 8 DHW have been shown to lead to widespread coral bleaching and habitat destruction in the tropics[41]. The accumulated thermal stress in corals, which begins at approximately 1 – 1.5 °C above the normal summer temperatures (following 4 – 6 week exposure) is consistent with the PT95 (1.4 °C) derived from our thermal SSD. The current study treats thermal stress like contaminants that may have multiple toxic pathways (e.g. metals). In some cases, this can result in taxon-specific modes of action and polymodal SSDs[14]; however, there was no apparent relationship between taxa and thermal sensitivity. While this dataset from 41 species across 12 phyla should represent a diverse array of sensitivities (and potentially thermal stress pathways) exhibited by a tropical marine community, future studies should aim to include an even broader range of species to improve confidence that the predicted proportion of species affected by temperature anomalies is a suitable representation of the community response. Importantly, the thermal SSD was generated from chronic exposure experiments that differ in duration between taxa and life stages as defined by the water quality guideline methods[31]. Therefore, the *PTx* values derived here represent a complementary approach to quantifying thermal stress thresholds for coral reef communities, and we suggest that the *PTx* values could be applied to a minimum of 2 week exposures (equivalent to the longer experimental exposures required by water quality GV derivation methods for chronic values[31]).

Thermal SSD. Thermal stress thresholds (*ET50*) have been shown to correlate with acclimation temperature (*Ta*) over wide temperature ranges, and are lower in species from tropical climates[25,42]. This is consistent with the *climate variability hypothesis* based on the decrease in annual SST variation range from temperate areas to the tropics, which results in coral reef ectotherms exhibiting smaller thermal windows and living closer to their thermal maxima[27,43,44]. *Ta* and *TTx* were not correlated for the tropical species dataset, likely due to a relatively narrow range of acclimation temperatures (22 °C — 29 °C), and this allowed us to employ *TTx* values directly as the x-axis of the SSD, and a single thermal SSD could therefore be applied across the narrow *Ta* range assessed. This thermal SSD approach could be developed and modified to suit different purposes. For example, selecting only data that applied summer acclimation temperatures (experiments where *Ta* = summer maximum) to derive *TTx* would generate thermal SSDs more compatible with other metrics for stress on coral reefs such as degree heating weeks (see above). The use of LT50 data (temperature above acclimation causing 50% mortality), which are less conservative than the ET10 and NOET data used in the current study, would generate thermal SSDs that predict the percentage of species seriously impacted (e.g. killed) by elevated temperatures (similar to previous thermal SSDs for mostly temperate aquatic species[25]). Furthermore, the use of thermal ranges from field data[23,24,38,45] could allow the generation of alternative thermal SSDs to to assess the tolerance of coral reef communities to heatwaves and address changes to community composition that might occur with the predicted increased frequency of heat waves as the climate changes[13].

Using ms-PAF to estimate the influence of thermal stress on water quality guidelines. Multisubstance-PAF has been used to predict the joint risk of multiple contaminants on aquatic communities from multiple SSDs[20,21]. Our study shows how the extension of ms-PAF to predict the simultaneous effects of chemical and physical stressors (in this case temperature) can be applied to adjust water quality GV to account for thermal stress. While the more direct approach of testing the toxicity of contaminants at many temperatures would be the ideal method to generate SSDs at thermally-stressful temperatures, such datasets are limited to only a few toxicants and individual taxa (see examples below). Other contaminant SSDs developed at different temperatures have so far been generated for species exposed within their normal thermal windows (e.g. tropical or temperate)[5,7,15,16], but do not generally account for the additional thermal stress as species encounter heatwave conditions. An advantage of the ms-PAF modelling approach is that it can draw upon the many existing contaminant SSDs, and accounts for the additional thermal stress by the development of a thermal stress SSD to predict the influence of elevated temperatures on toxicity.

Combining the thermal stress and contaminant SSDs using the IA joint action model resulted in reduced *PCx* values for copper and diuron as temperature increased; for example, predicted PC90 values were reduced between 29% (diuron) and 67% (copper) at temperatures of *Ta* +1.5 °C. This study illustrates that contaminant GVs are unlikely to provide their stated level of protection during heatwave conditions and will need to be decreased in order to do so. These thermal adjustments will become more important with the increasing frequency of heatwave events predicted under the ambitious goals of the Paris agreement [46], and more realistic scenarios [47]. These reductions in predicted concentrations of copper and diuron to provide a stated level of protection are not large because the temperature ranges being considered are relatively small (albeit ecologically relevant). As the temperatures increase to over 2.4 °C above acclimation, the thermal stress SSD predicts that more than 50% of the tropical reef community would have reached the thermal threshold, so temperature itself would dominate the negative effects on the community.

Validating the ms-PAF approach to predict the influence of thermal stress on contaminant toxicity. The application of ms-PAF_{IA} for any combination of contaminants assumes that distinct responses of individual species to each stressor (e.g. one species may be very sensitive to one class of contaminant, while another is more sensitive to another contaminant class) is ameliorated by combining high quality SSDs with many diverse taxa represented[17] (e.g. 41 species across 12 phyla for the thermal SSD). There are few laboratory data so far that can be used to effectively validate the ms-PAF approach. SSDs for temperate and tropical aquatic species (generated using data within their respective normal temperature ranges) have shown that PC90 (HC10) values could differ by up to 16-fold [5]. Another recent study derived SSDs for three metals from multiple single temperature tests (performed at different temperatures) and showed reductions in PC90 values by almost 50% when temperatures were increased by 2 °C intervals[7]. These studies and others that compare differences in sensitivity between temperate and tropical communities generally recommend that temperate water quality guideline values be divided by a 10-fold safety factor to derive guidelines for tropical species (for which there is generally little data)[6]. More specific validation of the ms-PAF predictions can be made by comparison with single species copper and diuron toxicity tests on tropical marine species, conducted at different temperatures. For example, the EC50s for larval settlement of two coral species to copper exposure were reduced by 19 and 27% (i.e. the corals became more sensitive) at *Ta* +3 °C[48]. Analysis of the same dataset showed that the IA model of joint action was able to accurately predict (to within 30%) the combined effect of most combinations of copper and temperature (up to *Ta* +3 °C)[28]. The IA model was also able to predict (<3 °C above *Ta*) the increase in diuron toxicity with elevated temperature in tropical coral symbionts, symbiont-bearing foraminifera and seagrass[36,37,49]. These single-species studies indicate that the IA model can be suitable for predicting joint effects of stressor combinations that include temperature. Reviews of the effectiveness of both the IA and chemical addition models to predict the impacts of multiple chemicals on aquatic organisms have revealed good agreement with observed responses, and that cases of underestimation (due to interactions) are rare[50,51].

Implications for risk assessment. The ms-PAF approach of predicting the combined effects of thermal stress and contaminants on tropical reef communities has several related applications for regulation and monitoring programs. For example, to maintain 95% protection of all species, during a heatwave the PC95 for any contaminant could be adjusted as shown in Table 2, where *Ta* is equivalent to the normal monthly average temperature. A similar approach could be taken to quantify the influence of water quality on heat stress to coral reef communities. For example, the thermal stress SSD predicts 10% of the community could be affected (=PT90) over prolonged heating of *Ta* +1.7 °C above the historical summer SST (*Ta*), and ms-PAF can be used to predict how contaminants change the proportion of the community affected. In addition, more than one contaminant can be included along with thermal stress in the ms-PAF[19]. Furthermore, the ms-PAF method could be applied to assess risk (spatially and temporally) by including thermal stress and contaminants as exposure layers in models and using SSDs and ms-PAF to model the potentially affected fractions of communities from individual locations and points in time. Extending this approach to temperate locations would require the generation of thermal SSDs for temperate marine species. SSDs for ocean acidification[52,53], sediment exposures[54], anoxia[55], and salinity[24,56] have already been developed for some freshwater and/or marine communities, offering further options for the ms-PAF approach to predict the hazards of multiple stressors in risk assessments and monitoring and reporting programs.

The generation of a thermal SSD for benthic marine organisms relevant to coral reefs presents an additional option for interpreting and quantifying the hazards posed by abnormally high sea surface temperatures that will become more frequent with predicted climate changes. Temperatures in tropical coral reef waters have already increased by nearly 1 °C since the industrial revolution[11], and present trajectories point more towards further SST increases > 1.2 °C by the end of the century[12]. Thus, the need for climate adjusted water quality guidelines is urgent. Understanding the combined hazards and risks posed by multi-contaminant mixtures is a developing field, with ms-PAF being one of the most widely explored "bottom-up" methods[57]. At present the influence of heatwave events on the risks posed by contaminants are either addressed by applying conservative (e.g. 10-times) safety factors or ignored. The extension of ms-PAF to include thermal stress is a promising approach that, with further development and validation, could enable the adjustment of water quality GVs to account for heatwave events that will help manage and protect coral reefs from the increasing frequency and duration of such events.

SUPPORTING INFORMATION: Composed of six appendices over nine pages. Figure S1: acclimation temperature *Ta* vs temperature threshold *TTx*. Figure S2: species sensitivity distributions (SSDs) for copper and diuron. Table S1: scoring system for assessing the quality of thermal stress data to benthic coral reef species. Table S2: Protection temperatures *PTx* calculated from multiple SSD distributions. Table S3: Thermal threshold values (*TTx)* and thermal acclimation values *(Ta)* for each species used to derive the thermal SSD. Table S4: SSD distribution parameters for the Burr type III distributions.

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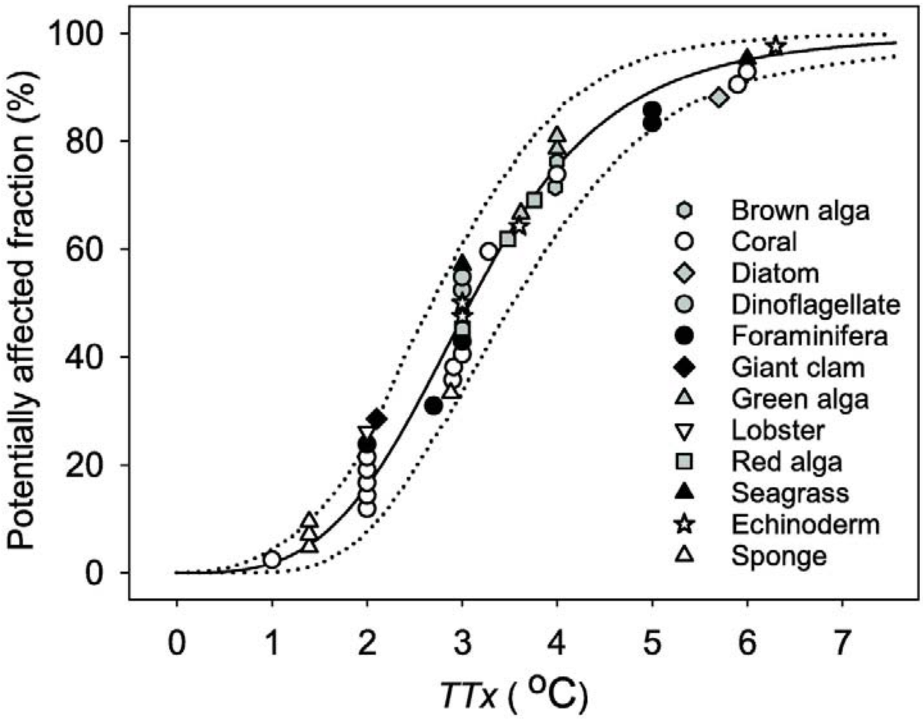

Figure 1. Species sensitivity distribution (SSD) of the thermal threshold data (TTx) used to derive protective temperature values (PTx). SSD was fitted with the Burr III function using Burrlioz 2.033 and replotted using SigmaPlot 13 (Systat Software Inc.). The dashed lines are the 95% confidence intervals for the SSD.

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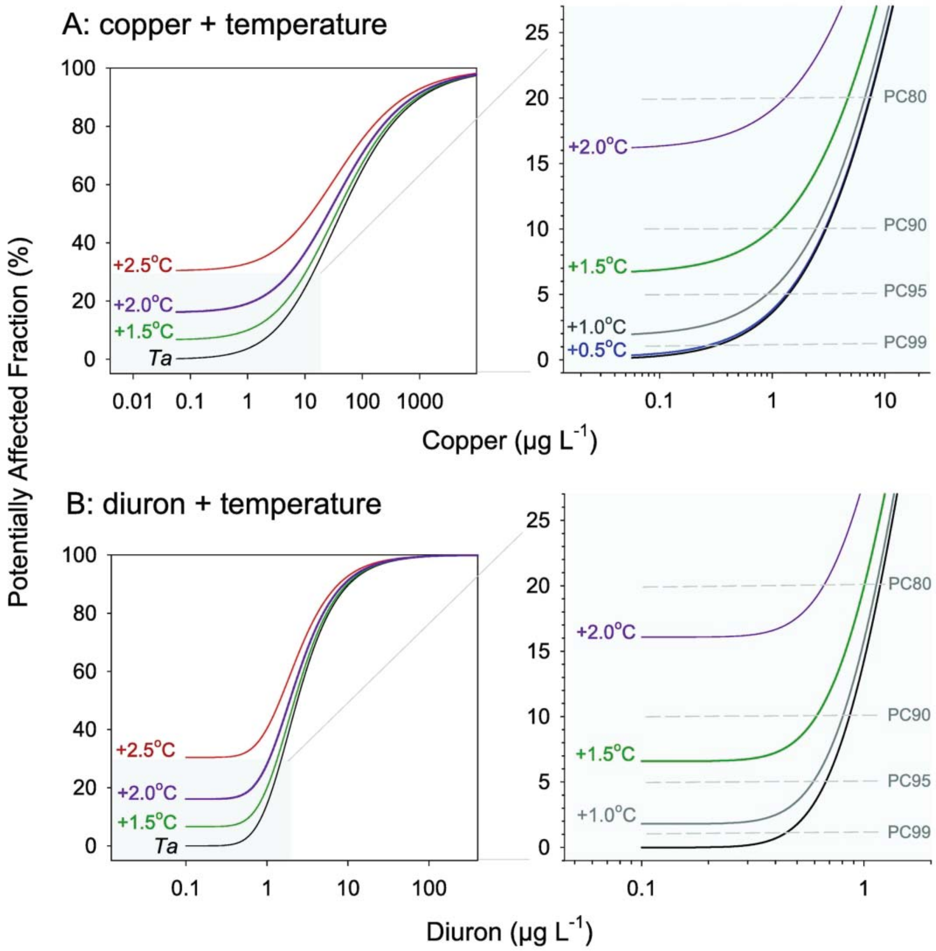

Figure 2. Species sensitivity distributions (SSDs) for copper (A) and diuron (B) at acclimation temperatures (Ta, black curve), and at temperatures greater than the acclimation temperature (TTx). The left plot shows full-scale SSDs while the right plot is an expanded view of grey areas in the left-hand plots. Adjustments to water quality guideline values (PC99 – PC50) are shown in Table 2.

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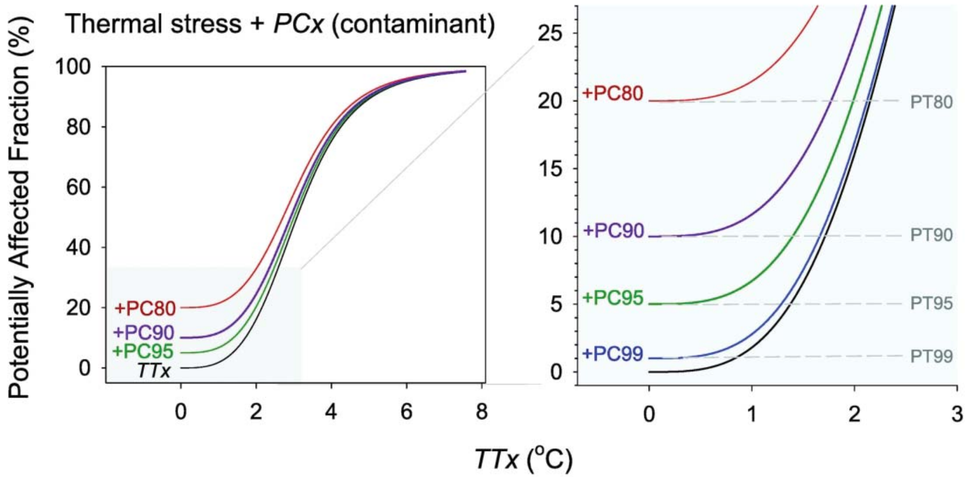

Figure 3. Species sensitivity distribution (SSD) for thermal stress at temperatures above acclimation (TTx) (black curve), and shifts in the thermal SSD at PC99, PC95, PC90 and PC80 concentrations for any contaminant (values for copper and diuron can be found in Table 2). The left plot shows full scale SSDs while the right plot is an expanded view of grey areas in the left-hand plots. Adjustments to suggested thermal stress guideline values (PT99 – PT50) as contaminant concentrations increase are shown in Table 1.

157x81mm (300 x 300 DPI)